

AMENDMENT AND RESPONSE TO OFFICE ACTION

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene corresponds to an inactivated native gene of the cell.

D²
3. (Amended) The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,

wherein the cell is a member of *Enterobacteriaceae*.

D³
23. (Amended) The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C,

wherein the cell is a member of *Enterobacteriaceae*.

D⁴
27. (Amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

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D4
Cord
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D4
(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment,

wherein the essential gene corresponds to an inactivated native gene of the cell.

29. (Amended) The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,
wherein the cell is a member of *Enterobacteriaceae*.

D5
30. (Twice amended) A method of inducing immunoprotection in a warm-blooded animal comprising
administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

D6
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(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,
wherein the essential gene corresponds to an inactivated native gene of the cell,
wherein the cell is a member of *Enterobacteriaceae*.

D6
35. (Amended) The method of claim 30 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a

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D⁶
correl. replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of *Enterobacteriaceae*.

Please add the following new claims.

41. (New) The cell of claim 1 wherein the essential gene is a gene essential for metabolism or growth of the cell.

42. (New) The cell of claim 1 wherein the essential is a gene essential for cell wall or cell membrane integrity.

43. (New) The cell of claim 1 wherein the essential gene is a modification methylase gene, a gene required for nucleic acid replication, or a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP.

D⁷ 44. (New) The method of claim 1 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab* gene, a *fad* gene, or a *pls* gene.

45. (New) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and